Effects of Kinetin, IAA, and Gibberellin on Ethylene Production, and Their Interactions in Growth of Seedlings[1, 2]

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Abstract. Kinetin in concentrations of 10^{-8} to 10^{-4} M, stimulated ethylene production in 3 and 4-day old etiolated seedlings of Alaska pea (*Pisum sativum* L. var. Alaska). Seedlings of other species responded similarly. The response to kinetin depended on the age of the seedlings.

Kinetin alone did not influence ethylene production in 6-day old stem sections, but it greatly increased the enhancing effect of IAA.

Gibberellic acid had no effect on ethylene production by pea seedlings during the first 6 days of growth. Ethylene and gibberellic acid are antagonistic in their effects on growth of the seedlings; ethylene interfered severely with the action of gibberellic acid but did not completely suppress it.

The inhibitors cycloheximide, cupferron, and *N*-ethylmaleimide, caused considerable inhibition of kinetin-induced ethylene production but were much less effective in the endogenous ethylene-forming system.

The influence of auxins on ethylene production in several plants was first reported by Zimmerman and Wilcoxon in 1935 (22). Recently several workers confirmed and extended this observation (1, 5) and currently there is an hypothesis which attributes many of the effects of auxin (IAA) to its influence on ethylene production (7).

Gibberellins have also been associated with ethylene production. For example, Abeles and Rubenstein (1) and Lewis, Palmer, and Hield (13) respectively, reported an increase of ethylene evolution by bean explants and parthenocarpic naval oranges, after treatment with gibberellins. Scott and Leopold (19), however, reported that the actions of ethylene and gibberellic acid are antagonistic in the elongation process of lettuce seedlings.

In contrast to IAA and gibberellins the relation of cytokinins to ethylene production and action has been studied much less. In a recent report on leaf abscission of bean explants Abeles, Holm, and Gahagan (3) noted that explants treated with high concentrations of kinetin and 6-benzyladenine doubled their ethylene production. We now present a study of the effects of kinetin on ethylene production, and the interactions between kinetin, IAA, and gibberellin on ethylene production and growth of seedlings.

Materials and Methods

Plant Materials. Alaska pea seeds (*Pisum sativum* L. var. Alaska) were soaked in a beaker in the dark, in running tap water bubbled with air. The soaking was carried out for 48 hr at 25°, a treatment which was found to produce uniform, disease-free seedlings. They were then planted on wet "Kimpack" (an absorbent of one-eighth inch thick packing paper) and grown at 25° and about 95 % relative humidity. The seedlings were grown in darkness up to 6 days with occasional dim "safe green" light. Seedlings were transferred to 50 ml flasks in the dark, at different ages, for the experiments. Age of seedlings was measured from the time the soaking started. In some experiments 2-cm apical sections were cut from 6-day old seedlings and used as experimental material. In experiments in which 1 day old seedlings were tested, the dry seeds were placed directly into 50 ml flasks, containing 2 ml of water or the test solution. Dwarf pea seeds (*Pisum sativum* L. var. Progress No. 9) were germinated in the same way.

In other experiments seeds of radish (*Raphanus sativus* L. var. Early Scarlet Globe), bean (*Phaseolus vulgaris* L. var. Tendergreen Improved), cucumber (*Cucumis sativus* L. var. Boston Pickling),

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wheat (*Triticum aestivum* durum L. em. Thell.), oat (*Avena sativa* L. var. Victoria), barley (*Hordeum vulgare* L. var. Himalaya), and corn (*Zea mays* L. var. Golden Bantam), were grown on wet filter paper in petri dishes for 48 hr, before being placed in 50 ml flasks for subsequent study of ethylene production.

Growth Regulators and Inhibitors. Kinetin (6-furfurylamino purine), 6-benzyl adenosine, adenine, IAA (3-indole acetic acid), gibberellin X (gibberellic acid potassium salt) and chloramphenicol, were purchased from Calbiochem[4]. *N*-ethylmaleimide (NEM) was purchased from Baker Chemical Company, 2,3,5-triiodobenzoic acid (TIBA) from Eastman Kodak, cupferron (*N*-nitrosophenyl hydroxyl amine) from Malinckrodt Chemical Company, and cycloheximide from Sigma Chemical Company. All chemicals were used as received without further purification.

Methods. All growth regulators and inhibitors were prepared in 10^{-3} M phosphate buffer, pH 6.8, and adjusted with 0.1 N KOH to pH 6.8 when necessary. The same buffer was used for controls.

In most experiments 2 ml of solution and 4 whole seedlings were placed in a 50 ml flask which was then sealed with a 1-hole rubber stopper, fitted with a clamped capillary tube. In some experiments 4 plumules, radicles, pairs of cotyledons or apical stem sections, 2 cm long, were used instead of whole seedlings. In all experiments the sealed flasks were held for 24 hr in the dark at 25°. The flasks were not shaken. At the end of the holding period the atmosphere in the flask was sampled with a syringe for ethylene determination.

Ethylene was determined by gas chromatography (16). A glass column, 75 cm long and 3 mm in diameter, containing activated alumina (70–80 mesh), to which 4 % water (by weight) was added, was used. Rates of ethylene production are presented on a fresh weight basis.

Unless otherwise indicated, at least 3 replications were run for each experiment. Statistical analysis was carried out using the analysis of variance and the Duncan Multiple Range Test (12) at the 1 % or 5 % level of significance.

Additional details of experimental methods are described in the text and in the legends of tables and figures where applicable.

Results

Effect of Kinetin Concentration on Ethylene Production. Kinetin in concentrations of 10^{-8} to 10^{-4} M stimulated ethylene production in 3-day old pea seedlings (fig 1). The rate of ethylene production increased with increased concentration of kinetin in the range of 10^{-7} to 10^{-4} M. Concentrations of kinetin greater than 10^{-4} M were not used because of solubility limitations at neutral pH. However, at pH 2.5, 10^{-3} M kinetin did not stimulate ethylene production in 3-day old pea seedlings. Therefore 10^{-4} M kinetin was the standard concentration used in subsequent experiments to produce

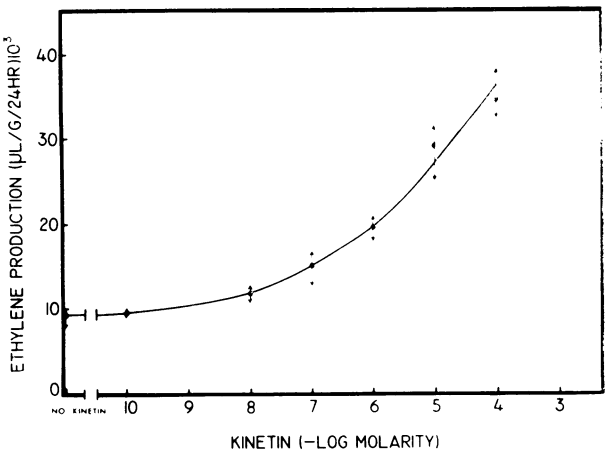

FIG. 1. Ethylene production induced by kinetin. Two-day old Alaska pea seedlings were grown at 25° in 50 ml sealed flasks containing 2 ml of 10^{-3} M phosphate buffer, pH 6.8, and various concentrations of kinetin. Each flask contained 4 seedlings. Ethylene production was measured after a growth period of 24 hr. Seedlings without kinetin, grown under similar conditions, served as controls. Range of results of 3 experiments are indicated by the double arrows at each point, which represents an average of the 3 experiments.

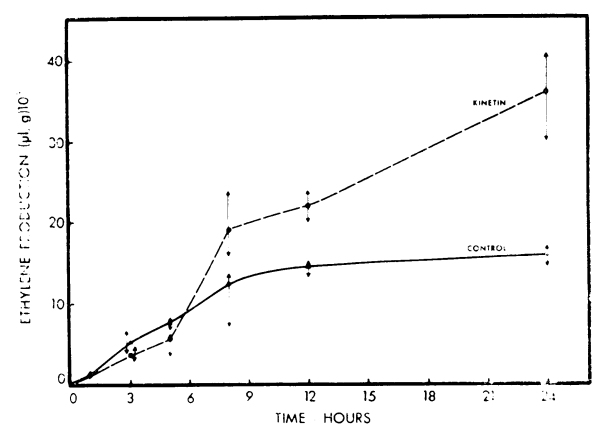

FIG. 2. Effect of 10^{-4} M kinetin on ethylene production. Groups of 4 etiolated 2-day old Alaska pea seedlings were held for different times up to 24 hr in 2 ml of 10^{-3} M phosphate buffer, pH 6.8, (control) or in 10^{-4} M kinetin in 10^{-3} M phosphate buffer, pH 6.8, (kinetin treatment). Each group was grown in 50 ml sealed flasks. The flasks were kept in the dark at 25°. Each flask was sampled only once. Range of results of 3 experiments are indicated by the double arrows at each point, which represents an average of 3 experiments.

[4] Mention of a specific trade name or company is made for identification only and does not imply endorsement by United States Department of Agriculture.

maximum ethylene production by seedlings. IAA-induced ethylene production in 6-day old pea-stem sections showed a similar "concentration-activity" curve (5).

Stimulation by Kinetin and Rate of Ethylene Production. Hourly production of ethylene in 2-day old pea seedlings, in the presence and absence of 10^{-4} M kinetin, is presented in figure 2. Three phases of ethylene production were observed; a slow phase (0–5 hr) in which production was about the same in both kinetin-treated and control seedlings; and ascending phase (5–8 hr) in which the greatest rate of increase was obtained, in both kinetin-treated and control seedlings; and a quasi steady-state phase (8–24 hr) in which ethylene production continued at a lowered steady rate in kinetin-treated seedlings, while ethylene production approached zero in controls. The rate of ethylene production of kinetin-treated seedlings was approximately 3 times and 5 times greater than controls in phases II and III respectively. The rate of ethylene production in phase III was considerably lowered in both kinetin treated and control seedlings. Part of this reduction in rate may be attributed to the accumulation of CO_2 in the sealed flasks. Carbon dioxide reached a concentration of 8 % during the 24-hr period, which inhibited kinetin-induced ethylene production about 12 %.

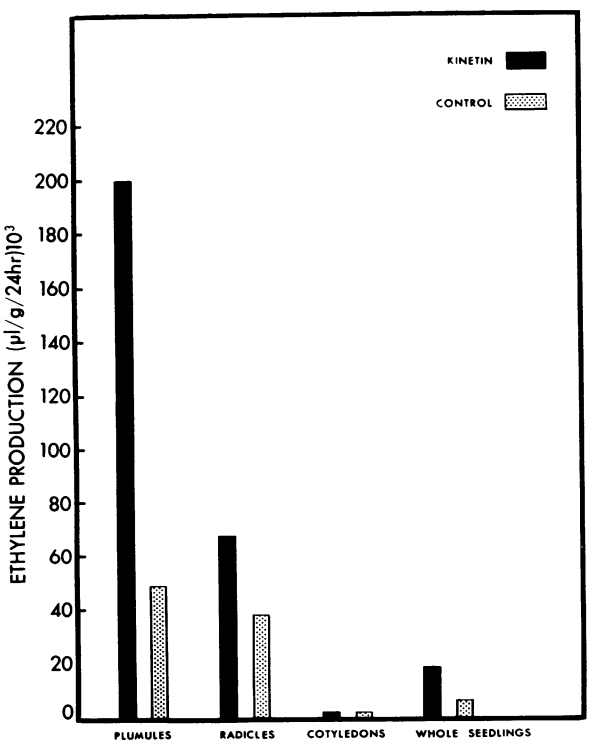

FIG. 3. Kinetin-induced ethylene production in radicles, plumules and cotyledons of Alaska pea seedlings. Three-day etiolated Alaska pea seedlings were separated by razor blade into radicles, plumules, and cotyledons. Four radicles, plumules, or cotyledons were incubated in the dark for 24 hr at 25° in 50 ml flasks with 2 ml of 10^{-4} M kinetin in 10^{-3} M phosphate buffer, pH 6.8, or 2 ml of 10^{-3} M phosphate buffer, pH 6.8 as the control treatment. Ethylene production is expressed on a fresh weight basis.

Effects of Kinetin on Ethylene Production by Radicles, Plumules, and Cotyledons. Studies of ethylene production by the 3 major morphological regions of 3-day old pea seedlings showed that ethylene production is different in each of these regions (fig 3). Plumules and radicles produce most of the ethylene while the cotyledons form virtually no ethylene. Addition of kinetin to these morphological regions of the seedlings resulted in considerable increase in ethylene production in radicles and plumules, but had no effect on cotyledons. Kinetin induced about an 80 % stimulation of ethylene production by radicals and about a 300 % increase in plumules. Thus only the actively growing regions which produce ethylene in relatively large amounts, are stimulated by kinetin.

Most of the weight of a 3-day old pea seedling is found in the cotyledons (400 mg) while the plumules and radicle contribute very little (25 mgs and 50 mgs, respectively). Therefore the specific ethylene production of the plumules and radicals appears very high, relative to the intact 3-day old seedling, which is made up mostly of non-ethylene forming cotyledons. The amount of ethylene produced on a per seedling basis by the 3 separated regions, was approximately the same as that produced by the intact seedling.

Effect of Kinetin on Ethylene Production in Seedlings of Several Species. Two-day old seedlings of radish, bean, cucumber, and corn were stimulated in ethylene production by 10^{-4} M kinetin (table I). Wheat, oat, barley, and Progress No. 9 (a dwarf pea variety) seedlings, however, were not significantly affected by the same kinetin treatment. These data suggest that kinetin does not influence ethylene production in monocots to the same extent as dicots. The lack of stimulation of ethylene pro-

Table I. *Ethylene Production by Seedlings of Several Species as Affected by Kinetin*

Groups of 4 seedlings, 2-days old, were grown in 50 ml flasks in 2 ml solution of 10^{-3} M phosphate buffer, pH 6.8, for the control, or 10^{-4} M kinetin in the same buffer for 24 hr at 25° in the dark. All seedlings were previously germinated on wet filter paper in petri dishes for 48 hr.

Species	Ethylene production	
	Control	Kinetin
	(*µl per g per 24 hr*) 10^3	
Radish	29.0	59.0
Bean	7.4	14.8
Cucumber	24.2	81.7
Pea var. Alaska	7.7	28.0
Pea var. Progress No. 9	10.5	10.2
Wheat	6.8	9.6
Oats	4.3	4.3
Barley	5.0	7.1
Corn	6.1	15.1

Table II. *Effect of Adenine, 6-Benzyladenosine, and Kinetin on Ethylene Production by Pea Seedlings*

Two-day old etiolated Alaska pea seedlings were grown in the dark in 2 ml solutions of adenine, 6-benzyladenosine, and kinetin at various concentrations in 10^{-3} M phosphate buffer pH 6.8, for 24 hr at 25°.

Cytokinin	None	Ethylene production Conc of cytokinins 10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
		(μl per g per 24 hr) 10^{3}				
Kinetin	11.6	29.8	21.0	12.1	13.1	15.5
6-Benzyladenosine	11.6	36.0	25.2	16.0	17.1	10.8
Adenine	11.6	15.5	13.7	15.5	15.5	15.5

duction in the dwarf seedlings (Progress No. 9) suggests that either kinetin does not influence ethylene production in the dwarf variety, or maximum ethylene production is occurring and additional kinetin cannot further influence the system already operating at its peak. The reaction of corn seedlings to kinetin is also not within the pattern shown by the other monocot seedlings tested.

Effect of Other Cytokinins. The cytokinin 6-benzyl adenosine stimulated ethylene production in 2-day old pea seedlings, much the same as kinetin (table II). However, adenine had no significant stimulatory effect on ethylene production of the pea seedlings. This agrees generally with the work of Skoog *et al.* (20) who assayed 69 purine derivatives and related compounds, for their cytokinin-like activity in the tobacco bioassay, and found adenine the least active of the substances tested.

Methionine as a Substrate for Kinetin-Induced Ethylene Production. Methionine is known to be a substrate for ethylene production in apples, bananas (6, 14), and pea seedlings treated with IAA (6). When 10^{-3} M methionine was added to 2-day old pea seedlings no increase in evolved ethylene was observed. However, when 10^{-4} M kinetin was added along with 10^{-3} M methionine, ethylene production increased about 250 % over controls and 60 % over the kinetin-treated peas (table III). The amount of ethylene formed by the pea seedlings treated with either kinetin plus methionine or IAA plus methionine, was about the same. Since methionine was shown to convert to ethylene in pea seedlings treated with IAA (6), it is probable that methionine is converted into ethylene in seedlings stimulated by kinetin.

Table III. *Kinetin-Dependent Stimulation of Ethylene Production by Methionine in Alaska Pea Seedlings*

Groups of 4 etiolated pea seedlings, 2-days old, were grown in the dark for 24 hr at 25° in 50 ml flasks. The flasks contained 2 ml of solutions of kinetin, methionine, or IAA in 10^{-3} M phosphate buffer, pH 6.8, as indicated. The actual amount of ethylene produced by the control was 0.013 μl per g per 24 hr. Numbers superscribed by no letters in common are significantly different at the 5 % level.

Incubation solution	Ethylene production % of control
10^{-3} M Phosphate buffer (control)	100[a]
10^{-4} M Kinetin	286[b]
5×10^{-3} M Methionine	100[a]
5×10^{-3} M Methionine + 10^{-4} M Kinetin	346[c]
5×10^{-3} M Methionine + 10^{-4} M IAA	340[c]

Effect of Inhibitors on Kinetin-Induced Ethylene Production. Enzymes involved in ethylene production appear to contain both a metal and sulfhydryl group in their active centers (14). NEM and cupferron, inhibitors of SH groups and metal ions respectively, significantly reduced kinetin-stimulated ethylene production in seedlings (table IV). Triiodobenzoic acid (TIBA), an inhibitor of IAA transport, and cycloheximide, an inhibitor of protein synthesis, also strongly inhibited kinetin-induced ethylene production. Chloramphenicol, an inhibitor of protein synthesis in bacterial systems but not in plant ribosomal systems (15), had no effect on ethylene production.

Table IV. *Effect of Inhibitors on Kinetin-Induced Ethylene Production*

Two-day old pea seedlings, in 2 ml of 10^{-3} M phosphate buffer pH 6.8, containing 10^{-4} M inhibitors, held for 24 hr in the dark in sealed flasks.

Inhibitor	Inhibition of endogenous system	Inhibition of kinetin-stimulated system
	%	%
TIBA[1]	23	65
Cupferron	3	71
NEM	28	86
Cycloheximide	10	100
Chloramphenicol	0	0

[1] TIBA = 2,3,5-Triiodobenzoic acid.

The inhibitors of ethylene production were considerably less effective against endogenous ethylene-forming systems than the kinetin-stimulated systems. This suggests that the kinetin-stimulated system is different from the endogenous system. In addition, the relative ineffectiveness of cycloheximide against the endogenous ethylene-forming system, and its complete inhibition of the kinetin-stimulated system supports the possibility that kinetin-induced ethylene

Table V. *Ethylene Production by Etiolated Pea Seedlings of Different Ages, in Presence of IAA, Kinetin, and Gibberellin, Alone and in Combinations*

Seedlings were grown and treated with growth substances as described in the text. In all treatments the final concentration of each of the participating growth substance was 10^{-4} M and pH 6.8. Kinetin, IAA, and gibberellin were dissolved in 10^{-3} M prosphate buffer pH 6.8. The experiments were replicated 4 times on 4 different days.

Days	Ethylene production							
	Control	Kn[1]	GA[2]	IAA	Kn + GA	GA + IAA	Kn + IAA	Kn + GA + IAA
	(μl *per g per 24 hr*) 10^3							
2	15	22	20	13	22	19	22	33
3	16	45	18	29	52	41	90	100
4	7	23	8	41	24	40	63	75
5	5	12	6	57	14	59	95	104

[1] Kinetin = Kn.
[2] Gibberellin = GA.

production is dependent on new enzyme formation in 2-day old pea seedlings.

Ethylene Production in Relation to Growth Substances and Age. Kinetin and IAA stimulated ethylene production in 3 to 5 day old pea seedlings, while gibberellin had virtually no effect (table V). During the first 2 days of growth there was little effect of any of the growth regulators on ethylene production by seedlings. On the third day the effect of kinetin was maximal and thereafter sharply declined. The stimulation by IAA, however, continued to increase linearly throughout the 5-day period. Thus the age of the seedlings was shown to be the controlling factor in eliciting increased ethylene production after treatment with IAA or kinetin.

Kinetin and IAA-treated seedlings were shorter and thicker than the controls, whereas gibberellin-treated seedlings were longer and somewhat thinner than the controls. This is to be expected from the effects of kinetin, IAA, and gibberellin on ethylene production of etiolated pea seedlings, and the known effects of gibberellin on elongation of seedlings. The increased ethylene production by seedlings treated with kinetin or IAA would tend to cause dwarfing, while treatment of the pea seedlings with gibberellin, which does not induce ethylene production, causes elongation.

When both kinetin and IAA were added to seedlings at the various ages, there was even greater ethylene production by the 3 to 5-day old seedlings (table V). However, when gibberellin was added to kinetin or IAA, or to combinations of kinetin and IAA, it did not significantly (statistically) enhance the stimulatory action of kinetin or IAA, or the combined stimulatory action of kinetin and IAA. Therefore, gibberellins had little or no effect on ethylene production by 3 to 5-day old etiolated pea seedlings, either alone or in various combinations with IAA and kinetin. On the other hand, kinetin and IAA significantly stimulated ethylene production in 3 to 5-day old etiolated pea seedlings when added alone, and their stimulatory action was enhanced by

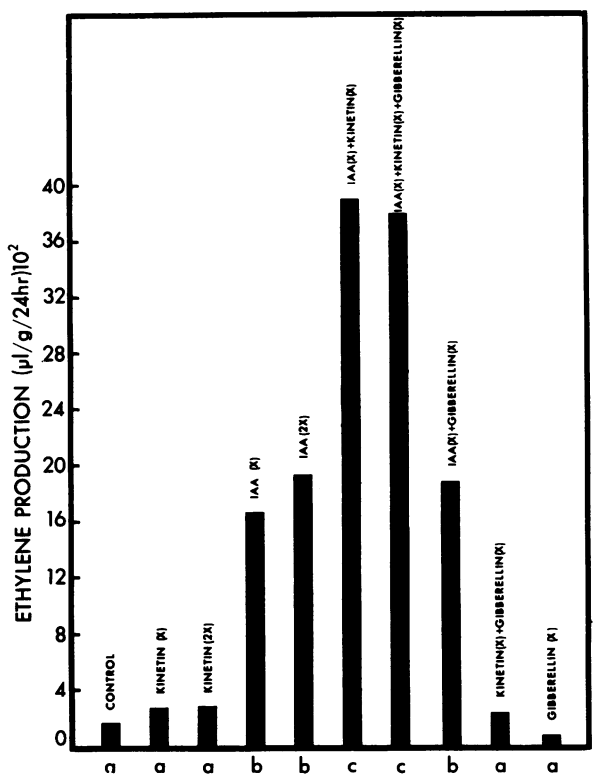

FIG. 4. Ethylene produced by 6-day old etiolated pea stem sections treated with kinetin, gibberellin, and IAA. Six-day old etiolated Alaska pea seedlings were grown as described in the Materials and Methods. Groups of 4 apical stem sections, 2 cm long, were cut with a razor blade and incubated in 50 ml sealed flasks for 24 hr, at 25° in the dark. Ethylene was determined at the end of a 24-hr period of growth. The flasks contained 2 ml of solutions of the different growth substances, each in final concentration of 10^{-4} M (X) or 2×10^{-4} M (2X) in 10^{-3} M phosphate buffer, pH 6.8. In the control treatment the stem sections were incubated with 2 ml of buffer only. Letters under columns on graph indicate Duncan Multiple Range Test (12). Columns on graph ascribed by no letters in common are significantly different at 1% level.

combining these 2 growth regulators in 1 treatment.

The combined action of growth regulators on ethylene production was also studied with 6-day old pea stem sections (fig 4). As previously noted (table V), kinetin did not greatly stimulate ethylene production in 5-day old pea seedlings, and also had little effect on 6-day old pea seedling stem sections (fig 4). However, when IAA was combined with kinetin considerable stimulation of ethylene production was obtained. IAA and kinetin interacted to increase ethylene production in stem sections from etiolated pea seedlings, even at an age (6-day old seedlings) when kinetin by itself had little effect on ethylene production. Gibberellin on the other hand had little or no significant effect on ethylene production, alone or in combination with IAA and kinetin, in either whole pea seedlings or their stem sections.

Ethylene-Gibberellin Interaction in Alaska Pea Seedlings. Inhibition of elongation of young pea seedlings is a well known effect of ethylene (5, 8, 17) and it has been shown that kinetin causes an increase in ethylene production in these seedlings. Therefore kinetin may cause inhibition of growth. On the other hand gibberellin stimulates elongation in Alaska pea seedlings in early stages of their growth (18), and we have already shown (fig 4, table V) that gibberellin has little or no influence on ethylene production. The interaction between ethylene and gibberellin was therefore investigated.

Pea seedlings grown in the dark for 4 days (15 mm height) were divided into 4 groups: I, non-treated seedlings (control); II, ethylene-treated seedlings; III, gibberellin-treated seedlings; and IV, seedlings treated with gibberellin on the fourth day followed by addition of ethylene on the fifth day. At the end of the seventh day of growth the jars of all treatments were opened (vented), to allow the escape of ethylene from the jars and the plants continued to grow in the dark for 48 hr, in normal room atmosphere, until the ninth day. Measurements of height (stem length) were made on the fifth, seventh, and ninth days of growth (table VI).

On the ninth day plants treated with gibberellin and ethylene (IV) were shorter than controls (I) and those treated with gibberellin alone (III), but taller than those treated only with ethylene (II) (table VI). Similar results were obtained on the seventh day when the stunting-effect of ethylene was even more obvious (table VI). On the seventh day the plants in treatment IV, containing gibberellin and ethylene, elongated about 50 % while those of treatment II, containing ethylene showed no elongation. The gibberellin treated seedlings (treatment III), however, were almost 3 times as tall as those in treatment IV.

After release from the ethylene atmospheres, the growth rates of treatments II and IV were not markedly different (table VII). However, treatment IV showed a somewhat higher growth rate, suggesting a residual effect of gibberellin, even 5 days after treatment. The growth rate of treatment IV, between the seventh and ninth days, was far below the rate for treatment III, which had been treated only with gibberellin. The action of gibberellin thus appears to be antagonistic to that of

Table VII. *Growth Rate of Seedlings Treated and Not-treated with Ethylene, Gibberellin, and Gibberellin Ethylene*[1]

Treatment		Rate of growth (stem length) 4th–5th day	5th–7th day	7th–9th day
		mm/hr	*mm/hr*	*mm/hr*
I	No treatment (control)	0.6	1.3	1.8
II	Ethylene-added on fifth day, vented on seventh day	0.6	0.0	1.3
III	Gibberellin-added on fourth day	0.6	1.9	2.3
IV	Gibberellin-added on fourth day, then ethylene added on fifth day, and vented on seventh day	0.6	0.3	1.6

[1] Data derived from table VI.

Table VI. *Ethylene-Gibberellin Interaction in Etiolated Alaska Pea Seedlings*

Numbers in the table represent the average height of at least 10 plants. Pea seeds were germinated in small plastic baskets in moist "Perlite" 16 seeds per basket. All baskets were placed in 4-liter jars to enclose the atmosphere. The experiments were done in the dark, with occasional "safe green" light for necessary manipulations.

Treatment		Stem length (mm) Day of growth Fourth	Fifth	Seventh	Ninth
I.	No treatment (control)	15	30	95	180
II.	Ethylene[1]-added on fifth day, vented on seventh day	15	30	30	90
III.	Gibberellin[2]-added on fourth day	15	30	120	230
IV.	Gibberellin[2]-added on fourth day, then ethylene[1]-added on fifth day, vented on seventh day.	15	30	45	120

[1] 2 μl per liter of ethylene was added.
[2] 5 μl 20 mg/liter gibberellic acid solution was added to the apices of the seedlings.

ethylene on elongation of pea seedlings. Ethylene severely limits but does not completely suppress the action of gibberellin.

Discussion

Ethylene production in 2 to 5 day old seedlings is influenced by cytokinins as well as by IAA. It also appears that there is an interaction between kinetin and IAA which regulates ethylene production in the very young seedlings. Since growth of meristematic tissue is known to be influenced by cytokinins (20), one may now wonder whether or not the ethylene induced by cytokinins plays a role in the action of cytokinins in young tissues. For example, the inhibition of elongation in seedlings by kinetin (4, 10, 11, 21) may be attributed to its stimulation of ethylene production.

Gibberellin on the other hand has no significant effect on ethylene production either alone or in combinations with IAA and kinetin, in 1 to 6 day old pea seedlings. In fact, the well-known action of gibberellin, which results in stem elongation, appears to be antagonized by the action of ethylene which induces dwarfing of seedlings. It is possible that interaction between kinetin, IAA, ethylene, and gibberellin, control elongation in seedlings. Antagonism between the elongation and dwarfing effects of gibberellin and ethylene was also reported by Scott and Leopold (19). In addition, another ethylene effect, the ripening of tomatoes, can also be antagonized by gibberellin (9). These data suggest that the actions of gibberellin and ethylene are mutually antagonistic in their effects on growth and development of tissues.

The increased production of ethylene induced by kinetin in these experiments is apparently not due to conversion of kinetin to ethylene, since greater stimulation of ethylene production was obtained when kinetin was added in the presence of methionine. This suggests that kinetin acts to stimulate the system which converts precursors to ethylene, rather than being itself converted to ethylene. Burg, and Clagett (6) suggested that IAA, which also stimulates ethylene production in seedlings, operates on the precursor system.

The fact that both IAA-stimulated ethylene production (2) and kinetin-stimulated ethylene production are inhibited by inhibitors of protein synthesis (table IV), suggests that these growth regulators induce ethylene production by affecting systems for protein synthesis. This may mean that new enzymes are needed to implement the production of the induced ethylene. Since the endogenous ethylene-forming system and the kinetin-induced system are inhibited differently by cupferron, NEM, TIBA, and cycloheximide, we may suspect that the induced ethylene-forming system differs from the endogenous system. However, before concluding that 2 kinds of ethylene-forming systems exist in young seedlings, we should consider that the inhibitors may react with kinetin or the kinetin-induced system causing interruption of the stimulatory effect.

It appears that ethylene formation in seedlings is directly interrelated with the important growth regulators kinetin and IAA, and is also indirectly related, in a complementary way, with gibberellin. A proper balance of these interrelationships may form the basis for regulatory control of growth and development in plant cells.

Acknowledgment

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Literature Cited

1. ABELES, F. B. AND B. RUBINSTEIN. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963–69.
2. ABELES, F. B. 1966. Auxin stimulation of ethylene evolution. Plant Physiol. 41: 585–88.
3. ABELES, F. B., R. E. HOLM, AND H. E. GAHAGAN. 1967. Abscission: The role of aging. Plant Physiol. 42: 1351–57.
4. BRIAN, P. W. AND H. G. HEMMING. 1957. Effects of gibberellic acid and kinetin on growth of pea stem sections. Naturwissenschaften 44: 594.
5. BURG, S. P. AND E. A. BURG. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Natl. Acad. Sci. 55: 262–69.
6. BURG, S. P. AND C. O. CLAGETT. 1967. Conversion of methionine to ethylene in vegetative tissue and fruits. Biochem. Biophys. Res. Commun. 27: 125–30.
7. BURG, S. P. AND E. A. BURG. 1967. Auxin stimulated ethylene formation: Its relationship to auxin-inhibited growth, root geotropism and other plant processes. Sixth Intern. Conf. Plant Growth Substances, Carlton University Book of Abstracts. p 11.
8. CROCKER, W., A. E. HITCHCOCK, AND P. W. ZIMMERMAN. 1935. Similarities in the effects of ethylene and plant auxins. Contrib. Boyce Thompson Inst. 7: 231–48.
9. DOSTAL, H. C. AND A. C. LEOPOLD. 1967. Gibberellin delays ripening of tomatoes. Science 158: 1579.
10. KATSUMI, M. 1962. Physiological effects of kinetin. Effect on the thickening of etiolated pea stem sections. Physiol. Plantarum 15: 115–21.
11. KATSUMI, M. 1963. Physiological effects of kinetin. Effect of kinetin on the elongation, water uptake and oxygen uptake of etiolated pea stem sections. Physiol. Plantarum 16: 66–72.
12. LE CLERG, E. L. 1957. Mean separation by the functional analysis of variance and multiple comparisons. U.S.D.A., ARS Publication 20–23.
13. LEWIS, L. N., R. L. PALMER, AND H. Z. HIELD. 1967. Interactions of auxins, abscission accelerators, and ethylene on abscission of citrus fruits. Sixth Intern. Conf. Plant Growth Substances, Carlton University Book of Abstracts, p 48.
14. LIEBERMAN, M., A. KUNISHI, L. W. MAPSON, AND D. A. WARDALE. 1966. Stimulation of ethylene production in apple tissue slices by methionine. Plant Physiol. 41: 376–82.

15. MARCUS, A. AND J. FEELEY. 1965. Protein synthesis in imbibed seeds. II. Polysome formation during imbibition. J. Biol. Chem. 240: 1675–80.
16. MEIGH, D. F., K. H. NORRIS, C. C. CRAFT, AND M. LIEBERMAN. 1960. Ethylene production by tomato and apple fruits. Nature 186: 902.
17. MICHENER, H. D. 1938. The action of ethylene on plant growth. Am. J. Botany 25: 711–20.
18. MOORE, T. C. 1967. Gibberellin relationships in the Alaska pea (*Pisum sativum*). Am. J. Botany 54(2): 262–69.
19. SCOTT, P. C. AND A. C. LEOPOLD. 1967. Opposing effects of gibberellin and ethylene. Plant Physiol. 42: 1021–22.
20. SKOOG, F., H. Q. HAMZI, A. M. SZWEYKOWSKA, N. J. LEONARD, K. L. CARRAWAY, T. FUJII, J. P. HELGESON, AND R. N. LOEPPKY. 1967. Cytokinins: Structure/activity relationships. Phytochemistry 6: 1169–92.
21. SOMMER, N. F. 1961. Longitudinal and lateral response of etiolated pea sections to indoleacetic acid, gibberellin, kinetin, sucrose and cobaltous chloride. Physiol. Plantarum 14: 741–49.
22. ZIMMERMAN, P. W. AND F. WILCOXON. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. 7: 209.